



## **Gliricidia sepium Aqueous Leaf Extract Possesses Antisickling Property**

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### **Authors' contributions**

This work was carried out in collaboration between all authors. Author TO designed the study, wrote the protocol and carried out the sickling test. Authors MKD and AOM wrote the manuscript. Authors MAN and SWH managed the literature searches and author IMA did the plant extraction. All authors read and approved the final manuscript.

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### **ABSTRACT**

**Background:** Extracts of leaf, flower and bark of *Gliricidia sepium* have been used in the treatment of patients with pathogenic bacterial infections, skin diseases, nematodes and antioxidants disturbances. We recently had information of its use in the treatment of sickle cell disease in some parts of Nigeria.

**Aim:** The aim of this study is to investigate the *in-vitro* antisickling properties of *Gliricidia sepium*

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aqueous leaf extract.

**Place and Duration of the Study:** This study was carried out in the Department of Chemical Pathology, Faculty of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.

**Methods:** Air-dried leaves were ground and an aliquot, 100 g was extracted by maceration in 1.0 L of distilled water as solvent for 72 h with periodic stirring. The same procedure was repeated with residues and the mixture was filtered and concentrated to dryness. Different concentrations (5 mg, 10 mg, 15 mg, 20 mg, 25 mg, 30 mg, 35 mg, 40 mg, 45 mg and 50 mg) were made from the extract and then tested for their antisickling activities against haemoglobin SS blood using freshly prepared 2% sodium metabisulphite.

**Results:** Our findings revealed strong antisickling activity; 20% antisickling at 5 mg, 50% at 10 mg, 80% at 15 mg and 100% antisickling from 20 mg upward for the leaf extract.

**Conclusion:** It has been shown for the first time, from this study that aqueous extract of *Gliricidia sepium* has strong antisickling properties justifying its use by the herbalists in the management of sickle cell disease. Toxicity studies, isolation and characterization of active compound are on-going in our laboratories.

**Keywords:** *Gliricidia sepium*; aqueous extract; antisickling; herbalist.

## 1. INTRODUCTION

The use of medicinal plants as a fundamental component of the African traditional healthcare system is perhaps the oldest and the most assorted of all therapeutic systems. In many parts of rural Africa, traditional healers prescribing medicinal plants are the most easily accessible and affordable health resource available to the local community and at times the only therapy that subsists [1]. The extensive use of traditional medicine in Africa, composed mainly of medicinal plants, has been argued to be linked to cultural and economic reasons. This is why the World Health Organization (WHO) encourages African member states to promote and integrate traditional medical practices in their health system [2]. Plants typically contain mixtures of different phytochemicals, also known as secondary metabolites that may act individually, additively, or in synergy to improve health. Indeed, medicinal plants, unlike pharmacological drugs, commonly have several chemicals working together catalytically and synergistically to produce a combined effect that surpasses the total activity of the individual constituents [1].

*Gliricidia sepium* is a leguminous tree and belongs to the family *Fabaceae*, originated in Central America, different parts of the plant is used in many tropical and sub-tropical countries for different purposes [3]. Phytochemical screening of the plant yielded a formosin (an isoflavan) with antitumor capacity, formononetin (an isoflavan), gliricidin-6a-gliricidinol-9a, medicarpin (a pterocarpan) with antifungal property, 7,4'-dihydroxy-3-methoxyisoflavin, 2'-O-

methylsepiol, tannin and a trihydroxyflavone [4]. Heartwood yielded a stigmastanol glucoside and 3', 4'-dihydroxy-trans-cinnamic acid octacosylester 2; two saponins (1 and 2), possessing 3beta, 21beta, 24-trihydroxy-22-oxoolean-12-ene as aglycon [4]. Extracts of leaf, flower and bark of *Gliricidium sepium* have been used in the treatment of patients with pathogenic bacterial infections [5], skin diseases [6,7], nematodes [8]; it has also been reported to possess antioxidants [9] and insecticidal properties [8].

Sickle cell disease (SCD) is a hereditary blood disorder caused by a single amino acid substitution (Glu---→Val) at the sixth position of the b-globin chains of haemoglobin. This single amino acid substitution causes a significant reduction in the solubility of the deoxy form of sickle haemoglobin (deoxy-Hb S), causing polymer formation inside the red blood cells [10,11]. Through a complex interplay of adhesive events among blood cells, these altered erythrocytes can obstruct the vasculature, producing episodes of pain, haemolytic anaemia, organ injury, and early mortality. Although the molecular basis of SCD is well characterized, the complex mechanisms underlying vasoocclusion (VOC) have not been fully elucidated [12].

The greatest burden of sickle cell anaemia (SCA) is in sub-Saharan Africa (SSA), where 75% of the 300,000 global births of affected children live, and estimates suggest that 50–80% of these patients will die before adulthood [13]. The WHO estimates that 70% of SCA deaths in Africa are preventable with simple, cost-effective interventions such as early identification of SCA

patients by newborn screening (NBS) and the subsequent provision of comprehensive care [14].

A lot of efforts had been made and are still being made to get treatment for sickle cell disease, especially drugs that will prevent sickling of red cells that usually precipitate crisis and hence ameliorate the excruciating pathological complications of the disease [15,16]. *Adansonia digitata* bark extract was documented to possess antisickling activities [17]. Antisickling activities of *Zanthoxylum heitzii* aqueous fruit extract [18], aqueous leaf extract of *Ocimum basilicum* [19], The use of aqueous leaf extract of *Gliricidia sepium* by herbalists in the management of sickle cell disease patients in some parts of Nigeria, initiated an interest to conduct a study to investigate the in-vitro antisickling properties of the aqueous leaf extract.

## 2. MATERIALS AND METHODS

### 2.1 Plant Collection and Identification

The leaves of *Gliricidia sepium* was collected from Osogbo, Osun State, Nigeria. The plant was identified and authenticated at the Herbarium unit of Botany Department, Obafemi Awolowo University, Ile Ife, by comparing with established Herbarium specimen with voucher number IFE/17460 which was deposited at the Herbarium.

### 2.2 Preparation and Extraction

Fresh samples of the leaves of *Gliricidia sepium* were collected and air-dried at room temperature. Air-dried leaves were ground and an aliquot, 100 g was extracted by maceration in 1.0 L of water as solvent for 72 h with periodic stirring. The same procedure was repeated with residues and the mixture was filtered and concentrated to dryness. The dried crude extract was stored in a refrigerator at low temperature (4°C) in sterile plastic bottles, at the Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, until required for use.

### 2.3 Blood Sample

Known haemoglobin (Hb) type AA and SS blood samples were obtained from the Haematology and Blood Transfusion Department, Usmanu Danfodiyo University Teaching Hospital, Sokoto and confirmed by carrying out Haemoglobin electrophoresis on Cellulose acetate paper at pH 8.4 on the blood samples.

### 2.4 Sickling Test

The principle of sickling test was based on microscopical observation of sickling of red blood cells when exposed to a low oxygen tension. In this method a drop of blood is mixed with a drop of sodium metabisulphite on a clean glass slide and cover slipped. The coverslip was gently pressed and the excess mixture was cleaned by tissue paper and edges were properly sealed with Vaseline jelly to prevent air from entering. Sodium metabisulphite is a reducing agent; it rapidly reduces oxyhaemoglobin to reduced haemoglobin to accelerate sickling. In positive samples the typical sickle-shaped red blood cells will appear [20].

### 2.5 In-vitro Antisickling Property of *Gliricidia sepium* Aqueous Leaf Extract

#### 2.5.1 Procedure

1. A drop of Hb SS blood was added to a drop of freshly prepared 2% sodium metabisulphite, mixed and cover slipped. The cover slip was gently pressed and the excess mixture cleaned with tissue paper. The edges of the cover slip were sealed using Vaseline jelly to prevent air from entering. The slide was placed in a moist chamber and incubated for 30 minutes at 37°C and examined under a Microscope at X10 and X40 objectives. It was incubated for another 30 minutes and examined as previously described. This served as a positive control [15].
2. The same procedure was repeated as described above but using Hb AA blood. This served as a negative control [15].
3. Ten different slides were prepared, a drop of Hb SS blood was mixed with a drop of freshly prepared 2% Sodium metabisulphite on each slide. Different concentrations: 5 mg, 10 mg, 15 mg, 20 mg, 25 mg, 30 mg, 35 mg, 40 mg, 45 mg and 50 mg of the crude dried extract were prepared using distilled water as solvent. A drop from each of these different concentrations was then added to each of the slides, mixed and cover slipped. The cover slips gently pressed and excess mixture cleaned with tissue paper, sealed with Vaseline jelly, and incubated and examined as described above. These slides assess antisickling activity of the extract [15].

**Table 1. Sickling test with Hb SS blood and freshly prepared 2% Sodium metabisulphite**

Sickling test positive control	
Volume of blood	1 drop Hb SS blood
Volume of 2% sodium metabisulphite	1 drop
Result	100 % sickling of red cells

**Table 2. Sickling test with Hb AA blood and freshly prepared 2% Sodium metabisulphite**

Sickling test negative control	
Volume of blood	1 drop Hb AA blood
Volume of 2% Sodium metabisulphite	1 drop
Result	No Sickling of red cells

**Table 3. Evaluation of antisickling property of *Gliricidia sepium* leaf extract**

Sickling test with Hb SS blood, freshly prepared 2% Sodium metabisulphite and the extract										
Volume of blood	1 drop of Hb AA blood									
Volume of 2% sodium metabisulphite	1 drop									
Concentration of the extract	5 mg	10 mg	15 mg	20 mg	25 mg	30 mg	35 mg	40 mg	45 mg	50 mg
Result	20%	50%	80%	100%	100%	100%	100%	100%	100%	100%

### 3. RESULTS AND DISCUSSION

Table 1 shows the result of Sickling test with Hb SS blood and freshly prepared 2% sodium metabisulphite. There was 100% sickling of the red cells (Positive control). *In vivo* sickling phenomenon was created in the sickling test. Sodium metabisulphite is a reducing agent, which reduced oxyhaemoglobin to deoxyhaemoglobin. Deoxygenated haemoglobin is less soluble, upon deoxygenation, haemoglobin precipitates and causes Hb S polymerization [21]. The Hb S polymers form an extremely viscous, solid like gel, which causes peculiar cell deformations, the most obvious of which is the sickle shape [21]. Because blood sample used in this test was sickle cell blood, sodium metabisulphite created low oxygen tension and induced sickling of red cells, and the red cells sickled.

Table 2 shows the result of Sickling test with Hb AA blood and freshly prepared 2% sodium metabisulphite. There was no sickling of the red cells (Negative control). Similarly, sodium metabisulphite created low oxygen tension but because the blood used was Hb AA blood, polymer was not formed and red cell did not sickle.

Table 3 shows the result of Sickling test with Hb SS blood and freshly prepared 2% sodium

metabisulphite and different concentrations of the extract. There was 20% antisickling at 5 mg, 50% at 10 mg, 80% at 15 mg and 100% antisickling from 20 mg up to 50 mg. Sodium metabisulphite also induced sickling here because Hb SS blood sample was used. The *Gliricidium sepium* leaf extract that was added to the blood – sodium metabisulphite mixture exhibited antisickling activities. This was evidenced by preventing about 20% of red cell from sickling at 5 mg, about 50% at 10 mg, about 80% at 15 mg and about 100% from 20 mg upward. This observation revealed antisickling property of the *Gliricidium sepium* leaf extract.

### 5. CONCLUSION

In conclusion, from the present study, our findings have revealed that *Gliricidium sepium* leaf extract possesses antisickling property. Although medicinal values of different parts of *Gliricidium sepium* have been widely documented, to the best of our knowledge, this is the first time we are reporting antisickling properties of *Gliricidium sepium* leaf extract, hence justifying its use by the herbalist in the management of sickle cell disease patient. Toxicity studies in animal, isolation and characterization of bioactive compound(s) are on-going in our laboratories.

## CONSENT

It is not applicable.

## ETHICS

It is not applicable.

## DISCLAIMER

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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